Cadernos Lab. Xeolóxico de Laxe
Coruña. 1999. Vol. 24, pp. 73-87

Paleoecological implications inferred from stable isotopic signatures ($\delta^{13}C$,$\delta^{15}N$) in bone collagen of *Ursus spelaeus* ROS.-HEIN.

Implicaciones paleoecológicas inferidas de la caracterización isotópica ($\delta^{13}C$,$\delta^{15}N$) del colágeno óseo de *Ursus spelaeus* ROS.-HEIN.

VILA TABOADA, M.; FERNÁNDEZ MOSQUERA, D.; LÓPEZ GONZÁLEZ, F.; GRANDAL d'ANGLADE, A. & VIDAL ROMANÍ, J.R.

ABSTRACT

Stable isotopic signatures measured in bone collagen provide with data related to the species diet type. In this paper we compare $\delta^{13}C$ and $\delta^{15}N$ outcomes in *Ursus spelaeus* ROS.-HEIN. bone remains from Liñares site and Cova Eirós site (Galicia, NW of the Iberian Peninsula). Some data on fossil *Ursus arctos* L. and Pleistocene *Cervus elaphus* L. from Galician caves are also presented, as a first approach to distinguish paleodiets of different species inferred from their isotopic signatures. Once all data have been analyzed with proper statistical tools and since this work was planned in order to reduce variation in stable isotopic signals caused by metabolic causes, we may assume that the observed differences between both studied groups are exclusively due to environmental factors and show a migration of the cave bear population in the *Serra do Courel* mountains from higher to lower altitudes because of the transition from warm climatic conditions to colder ones.

Key words: $\delta^{13}C$, $\delta^{15}N$, collagen, paleodiets, *Ursus spelaeus*, *Ursus arctos*, *Cervus elaphus*, paleoclimatology, Late Pleistocene, Galicia, NW Spain.

Instituto Universitario de Xeoloxía *"Isidro Parga Pondal"*. Universidade da Coruña. Facultade de Ciencias. Campus da Zapateira s/n. 15071. A Coruña. Galicia. Spain. *http://www.udc.es/dep/geda*

INTRODUCTION

This work goes on with isotopic biochemistry research on Quaternary fossils from Galician sites, started (FERNÁNDEZ, 1998) with *Ursus spelaeus* ROS.-HEIN. from Cova Eirós. In this paper we show first outcomes on *U. spelaeus* from Liñares site and even some data from *Cervus elaphus* L. and *Ursus arctos* L. We are going to tackle the reconstruction of paleoenvironmental changes inferred from isotopic data got from fossil bone remains. This kind of studies -recently started (FERNÁNDEZ, 1998) on Galician sites- have proved to be a proper tool in helping the reconstruction of ancient environments when those are coupled with the most common geological data, as sediments or geomorphology (GRANDAL et al., 1997). In the last years, the knowledge about Quaternary Period in Galicia (VIDAL ROMANÍ, 1996) has undergone a strong impulse due to a multidisciplinary approach and the use of highly specialized techniques, which contribute to solve the problem of understanding a period of time so scarce in fossil data as happens in Galicia.

The objetive of this work is to determine what kind of paleoenvironmental conditions surrounded the occupation of sites such as Liñares and Eirós (GRANDAL & LÓPEZ, 1998; LÓPEZ & GRANDAL, 1998) with an important record of Pleistocene macrofauna. Both are considered as different steps in the migration process of populations happened during glacial terms.

Isotopic signals are thought as important tools in order to supporte the hypotesis of paleoclimatic changes in late Pleistocene in Galicia. Such a theory has been proposed from geological data related to glaciar phenomena (VIDAL ROMANÍ, 1996), faunistic assemblages found in different sites (GRANDAL et al., 1997) and some modern techniques for dating rock surfaces (FERNÁNDEZ, 1999).

The meaning of stable isotopic signatures preserved in fossil animal tissues can vary depending on metabolic or environmental causes. Our attention will be focused in the cave bear, extinct aproximately 15,000 years ago and with a well known trend towards herviborism (KURTÉN, 1976). Then, the aim of this paper will be to analyse isotopic signals of *Ursus spelaeus* bone collagen in order to distinguish whether a qualitative climatic change existed or not between the ages when those sites were occupied; taking into account the influence of climate in the isotopic fractionation along the trophic chains.

As stable isotopic outcomes from carbon and nitrogen signals in fossil bones have been used as paleoenvironmental markers, it should be possible to compare outcomes with close and distant contemporaneous individuals data.

Often, teeth and bones are the only remains that survive in the fossil record, but as they are not closed systems, they can suffer weathering, infiltration and quite enough damage because of several process after death; so, a critic step in any study will be to evaluate collagen preservation. Thus, a suitable C/N atomic value (between 2,9 and 3,6 after DE NIRO,1985) will be required. During

diagenesis, C/N values can raise due to deamination of aminoacids or invasion by soil humic acids, and even fall because of inorganic material contamination (TURBAN-JUST & SCHRAMM, 1998). By proving that isotopic signals are original, and taking into account that collagen damage is not dependant on time, but fossil preservation conditions (TUROSS & STATHOPLOS, 1993), isotopic data will be able to be used as markers of paleoenvironmental conditions.

Stable isotopic compositions of food and drinks of animals have a strong influence on the isotopic compositions of the tissues they synthesize. Conversely, the isotopic composition of animals tissues can serve as a natural tracer of different dietary inputs with distinct isotopic signatures (DE NIRO, 1985). However, the exact relationship between the isotopic compositions of ingested materials and those associated to any particular tissue or molecular component is quite complex, responding not only to changes in nutritional status, but turnover rate of the tissue and biosynthethic pathway. Then, stable isotope analysis provides more than just a tracer of the materials that go into an animal, but it offers a view of the biological processes within an organism (KOCH et al., 1994) and thus, some clues to reconstruct the environment where it lived.

As many authors have noted, the atomic ratio between heavy isotopes and lighter ones ($R_x = {}^mX/{}^nX$, being m>n) is compared with a reference control. Delta symbol (δ) means the difference between sample R and the control one (LAJTHA & MICHENER, 1994). So:

$$\delta(‰) = [(R_{sample} - R_{reference})/ R_{reference}]\cdot 1000$$

Carbon and nitrogen stable isotope signatures may differ depending on some factors. The former element is influenced not only by isotopic composition of atmospheric CO_2 influences, but also by photosynthesis type (C_3 or C_4) in the basement of trophic chain too. Thus, plants with Calvin cycle (C_3) absorb less ^{13}C from CO_2 than Hatch-Slack (C_4) ones. Then, alimentary chains based in a vegetal step with C_3 as dominants will be depleted in the heavy isotope, which -according to logical fractionations at the same time we get higher levels- make animal tissues to have minor $\delta^{13}C$ values. We should remark that C_3 plants are associated with temperate or boreal climates, excluding tropical conditions which are supposed for C_4 elements. Moreover, other factors as species, physiology -example of lipidic catabolism in ursids during hibernation (ANDERSON, 1992)- and kind of tissue might affect these values (AMBROSE & DE NIRO, 1989; BOCHERENS et al., 1991a; KOCH et al., 1994; LAJTHA & MARSHALL, 1994).

Dealing with nitrogen signal, inputs of heavy isotope are determined by atmospheric N_2 fixation by micoorganisms in symbiosis with some particular plant species. Thus, fixator plants display a lower $\delta^{15}N$ than non-fixators. Several factors affect fractionation process and enrichment when considering higher trophic steps in the alimentary chain: soil conditionants as humi-

dity, acidity and age, animal physiology, specific metabolism pathways for nitrogen, kind of tissue, suckling in mammals and, only in high vegetal density forest, the canopy effect (AMBROSE, 1991; BOCHERENS et al, 1991a; BOCHERENS et al., 1991b; NELSON et al., 1975; CORMIE & SCHWARCZ, 1994).

Sample and Techniques

A sample of 38 bone remains from several Galician sites was assembled. It has been used 23 cave bear (*Ursus spelaeus*) ribs, and the vertebra - Lus24- and metapod -Lus25- used on ancient DNA studies (VILA, 1998); all of them came rom Liñares site (Galicia, NW of Iberian Peninsula) Furthermore, a metatarsian - Lce1- and 6 ribs from deer (*Cervus elaphus*) of Liñares site were also added, as well as 8 ribs of Holocene brown bear (*Ursus arctos*) from Tarelo and Purruñal sites (Galicia, NW Iberian Peninsula). All selected bones belonged to adult individuals.

Liñares site (LÓPEZ, 1996; LÓPEZ et al., 1997; GRANDAL & LÓPEZ, 1998) is a small karst cave, located in the *Serra do Courel* mountains, at 1,115 m above sea level (Fig. 1).

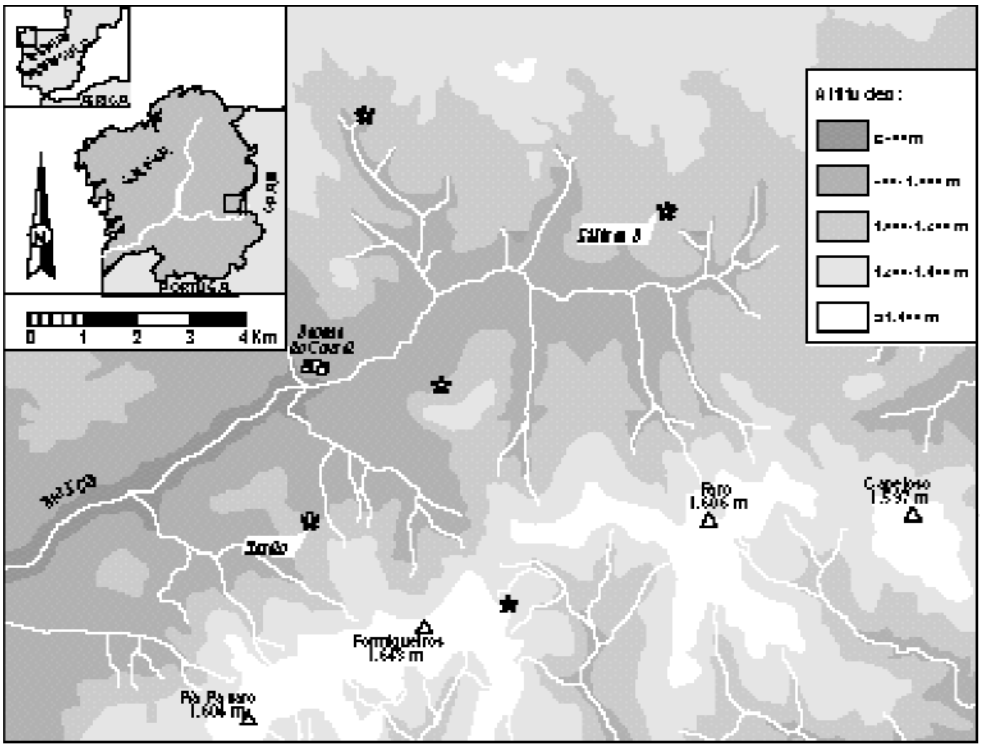


Figure 1.- Geographical location of Liñares site.

Figure 1 plots the location of Liñares (LÓPEZ & GRANDAL, 1998; GRANDAL & LÓPEZ, 1998) and Tarelo site, where an *Ursus arctos* skeleton comes from. The age of the mentioned sites has been inferred from absolute datings carried out on bone fossil remains preserved on their sediments. Thus, Tarelo site occupation is assumed during Holocene term due to geomorphological data and a brown bear bone dated -by ^{14}C method- in 7,460 ± 95 yBP (GRANDAL et al., 1997). Liñares site is supposed to be active around 35-40,000 yBP, this is inferred from a *Cervus elaphus* bone dated around 37,000 yBP (LÓPEZ, 1996; GRANDAL et al., 1997) and age outcomes for *Ursus spelaeus* bone remains from Liñares were 35,000 ± 1,440 yBP and >38,000 yBP (GRANDAL et al., 1997; VILA, 1998).

As %N is a first indicator about collagen preservation, bone powder (got after sewing and crashing the bone) was analyzed in a Carlo-Erba 1108 Elemental Analyzer with analytical reproducibility better than 0.1%. Once it was rejected those bones not suitable enough for isotopic determination due to their minimum bone %N (IACUMIN et al., 1997), we followed a common extraction method for collagen (BOCHERENS et al, 1997b) based on some digestions with HCl and NaOH and filtrations, in order to remove carbonates, humic contaminants and make soluble the extraction product. Afterwards, this material was freezed-dried and analyzed by SIRMS (Stable Isotopic Ratios Mass Spectrometry). Collagen isotopic signals for carbon and nitrogen were measured in a Finnigan Mat Delta Plus joint to a Elemental Analyzer Carlo-Erba 1108 with analytic reproducibility better than 0.1‰ for carbon and 0.2‰ for nitrogen. Outcomes are referred to international standars PDB and atmospheric N_2.

Taking into account that well preserved collagen, from isotopic point of view, would yield an atomic C/N ratio between 2.9 and 3.6 (DE NIRO, 1985) we must reduce the initial number of 38 bone samples to 30. Proportions for each site are shown in figure 2, whereas final outcomes are shown in table 1.

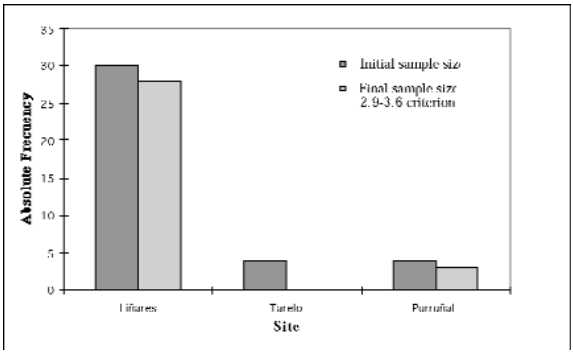
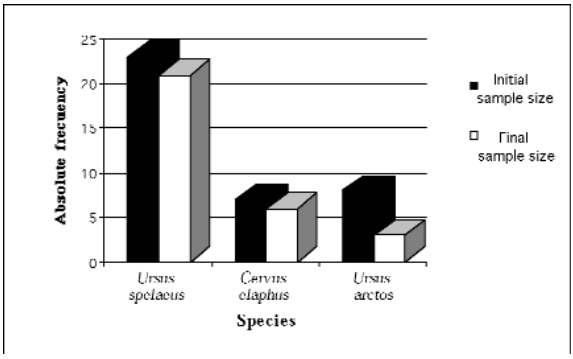


Figure 2.- Sample size of the studied bones depending on site (a) and species (b). Final sample size is reduced due to the 2.9-3.6 criterion for collagen preservation (DE NIRO, 1985).



Results

Sample	Bone % N	Yield mg/g	%N col	%C col	Atomic C/N col	$\delta^{15}N$ col	$\delta^{13}C$ col
Lus1	0.79	70.40	5.70	15.60	3.17	3.04	-21.24
Lus2	2.61	70.63	9.12	24.69	3.14	3.49	-21.23
Lus3	2.44	219.14	8.85	23.99	3.14	3.24	-21.14
Lus4	2.26	111.94	9.07	24.42	3.12	3.35	-20.65
Lus6	3.04	78.48	11.80	32.16	3.16	3.35	-20.86
Lus7	2.35	85.12	7.41	20.71	3.24	3.56	-21.50
Lus8	3.09	98.86	10.34	28.40	3.19	3.12	-20.87
Lus9	2.52	88.57	12.10	32.73	3.14	2.93	-20.97
Lus10	2.09	30.87	13.19	35.51	3.12	3.20	-20.90
Lus11	2.67	50.54	11.64	30.74	3.06	2.96	-21.28
Lus12	2.64	112.82	13.45	36.20	3.12	2.81	-20.63
Lus13	0.55	35.45	8.53	17.69	3.07	3.11	-21.41
Lus14	0.59	36.30	7.86	20.45	3.02	2.15	-21.13
Lus16	3.06	92.88	8.80	23.92	3.16	3.18	-20.97
Lus17	2.33	55.08	10.25	27.89	3.16	3.39	-21.72
Lus18	3.02	86.31	9.98	25.77	3.00	2.17	-20.92
Lus19	2.48	73.16	10.83	30.21	3.24	3.61	-20.92
Lus20	3.01	67.43	11.93	31.95	3.11	3.05	-20.77
Lus24	2.68	127.68	7.55	20.49	3.15	2.92	-21.23
Lus25	3.07	229.08	9.07	24.41	3.12	2.11	-20.94
Lce2	2.76	59.06	6.40	17.20	3.12	5.88	-19.65
Lce3	2.69	83.14	12.33	33.15	3.12	7.42	-19.92
Lce4	2.85	56.81	6.64	18.17	3.18	5.71	-20.10
Lce5	1.94	49.09	10.31	27.37	3.08	6.19	-19.72
Lce6	2.98	67.92	10.86	29.17	3.12	6.18	-20.00
Lce7	3.00	59.14	10.53	28.27	3.11	6.65	-19.77
Pua1	3.05	139.39	12.01	32.64	3.15	5.21	-20.00
Pua2	2.62	143.26	13.94	36.95	3.08	5.40	-19.54
Pua3	2.43	111.05	12.79	32.52	2.95	4.94	-19.41

Table 1.- Outcomes from 30 bone samples. L (first letter from site as figure 2, i.e.: Liñares), us (initial of species, i.e.: Ursus spelaeus).

According to previous works (BOCHERENS, 1997; CORMIE & SCHWARCZ, 1994), isotopic signals allow to distinguish different species. Following those references we can ensure that isotopic signals for the *Cervus elaphus* and *Ursus arctos* remains included in this paper are feasible. (Fig. 3).

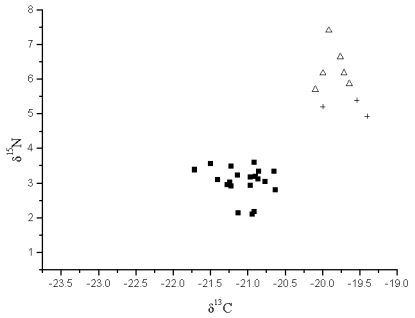


Figure 3: Isotopic signatures outcomes for Ursus spelaeus ( ), Cervus elaphus (³) and Ursus arctos (+).

We have chosen a Kruskal-Wallis test, which works with sample median instead of the mean, in order to resolve whether differences in $\delta^{13}C$ are significant. Results are shown in figure 4.

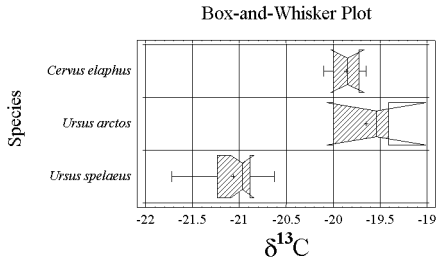


Figure 4.- Significant differences in $\delta^{13}C$ depending on species. Cave bear values are really different from deer and brown bear ones: p-value 0.00014. Kruskal-Wallis test results agree with ANOVA-I. Segments show the interval (95% confidence) got for sample mean (+). Notch sets median position.

Another Kruskal-Wallis test proved that differences for $\delta^{15}N$ were significant depending on species: p-value < 0.05. So, our 30 data agree with trophic levels for these species (IACUMIN et al., 1997; BOCHERENS, 1997).

Focusing in *Ursus spelaeus* isotopic signatures, we should remark the difference in $\delta^{15}N$ depending on age stage (FERNÁNDEZ, 1998). Data from Eirós non-suckling individuals will be plotted in figure 5 together with Liñares cave bear in order to distinguish differences in nitrogen signatures

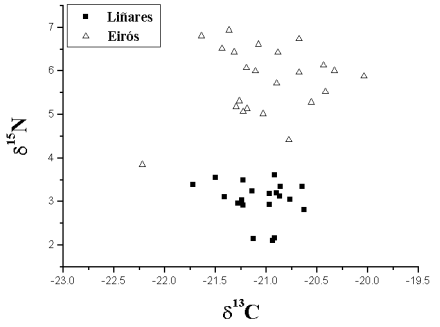


Figure 5.- Carbon and nitrogen stable isotopic signals of Ursus spelaeus bone collagen. $\delta^{15}N$ indicates two separate groups depending on the site.

We can appreciate that Liñares data are close grouped. $\delta^{13}C$ variation among individuals is not higher than ± 0.5 ‰ nor $\delta^{15}N$ one is higher than ± 0.6 ‰ (WANG & CERLING, 1994). Thus, inside each population, variation is caused by metabolic parameters, whilst variation among different populations would be due to environmental ones.

A *t-Student* test for $\delta^{13}C$ depending on site, Eirós and Liñares, shows no significant differences (p-value < 0.05). A U-Mann-Whitney for $\delta^{15}N$ let us reject (p-value < 0.0001) the null hypothesis of similar medians between Eirós and Liñares data (see figure 5).

As the extraction product is true collagen, with a C:N ratio close to 3, it can be seen in figure 6 that the regression line got from Liñares bones (cave bear and deer) shows a slope close to the proposed value. Brown bear remains from Purruñal site fit properly to this linear trend. Determination coefficient, R_2, also agree with the hypothesis of collagen as extration product: 96.07 % of sample variability can be explained by a linear regression.

Whereas, the Eirós equation for the same parameters was:

Y = 2.52 X + 1.402; Pearson r= 0.991 (FERNÁNDEZ, 1998).

By testing -with a paired *t-test*- the null hypothesis of non difference between collagen %C mean from Eirós to Liñares, we got a p-value = 0.57, and we cannot reject such a null hypothesis. Same fact was obtained for collagen %N. It can be concluded that a time difference of 13,000 years between Eirós (25,000 yBP aprox) and Liñares (38,000 yBP aprox) remains has not affected to collagen preservation.

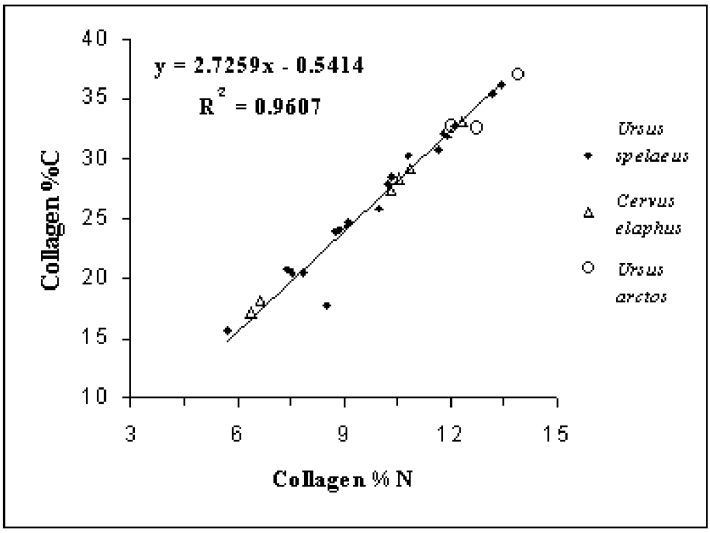


Figure 6: Carbon and nitrogen percent in extraction product. Linear regression trend line for Liñares (Ursus spelaeus and Cervus elaphus bones) explains 96% of the sample variability, slope close to 3.

DISCUSSION

This paper integrates very different information: isotopic biochemistry, metric palaeontology, absolute dating, and geomorphology with the final result of attaining paleoclimatic reconstructions.

Focused in *Ursus spelaeus* from Galician sites, we use the non-suckling group from Eirós Cave (FERNÁNDEZ, 1998) as isotopic reference. As we have reduced the metabolic causes of variation by using the same species, same kind of tissue and non suckling individuals, we could blame

environmental parameters as origin of isotopic differences.

In figures 4 and 5, cave bear shows the lowest $\delta^{15}N$ and $\delta^{13}C$ values among herbivores (BOCHERENS, 1997; BOCHERENS et al., 1997a). Its low isotopic signal in bone collagen relates with diet, climate and physiology,(AMBROSE, 1991). $\delta^{13}C$ from Liñares -as Eirós (FERNÁNDEZ, 1998)- is consistent with an alimentary chain based in C_3 plants, under humid/temperate conditions (BOCHERENS et al., 1991b; BOCHERENS et al., 1997b).

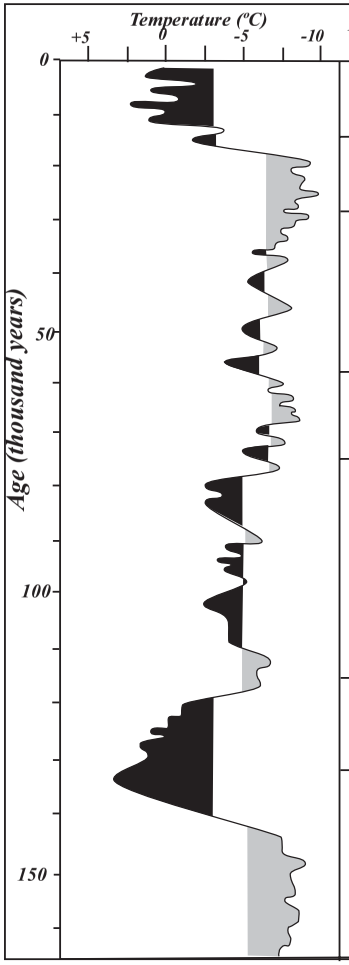
Nutritional and hydric stress are important conditionings for $\delta^{13}C$ and $\delta^{15}N$ isotopic signals. In order to avoid the influence of metabolic parameters, it is really crucial to keep in mind different requirements as: choosing the same species, tissue and age stage. Nevertheless, some previous works (see table 2) have used time intervals too large and thus, influence of metabolic parameters cannot be discriminated.

In this work, Liñares data (occupation around 38-40,000 y BP and 1,115 m above sea level), and Eirós ones (around 25,000 y BP and 715 m a.s.l.) have been compared. It must be granted that, once eliminated the metabolic influence in $\delta^{15}N$, differences in this signal (between Liñares and Eirós) correspond exclusively to environmental factors.

SITE	$\delta^{13}C$	$\delta^{15}N$	Age	n
1. Eirós (suckling)	[-22.79 to -21.06]	[5.16 to 9.96]	*	25
2. Eirós (non suckling)	[-22.22 to -20.44]	[3.85 to 6.94]	24,090 ± 440 yBP ζ	23
3. Liñares	[-21.72 to -20.63]	[2.11 to 3.61]	> 38,000 yBP ζ 35,000 ± 1,440 yBP) ζ	20

Table 2: Intervals for Galician cave bear $\delta^{13}C$ and $\delta^{15}N$ values. (1, 2) FERNÁNDEZ, 1998. [n] Sample size. [ζ] Datings by ^{14}C AMS bones. [*] Same stratigraphic level that non suckling, so it is assumed the same age that the Eirós non-suckling group.

According to previous literature, there is a good correlation between low $\delta^{15}N$ values and forestry habitats (BOCHERENS et al., 1994). Lower values for $\delta^{15}N$ (as Liñares) are related to cool and wet conditions. As the assumption of a dense forest, with cold and humid soils, is made from a present point of view, it would be naïve to suppose that Liñares individuals lived in a colder phase than Eirós inhabitants. Initially, Liñares and Eirós sites are so close that would not seem very real to establish strong climatic differences between these two sites. Then, $\delta^{15}N$ differences between Eirós and Liñares (see figure 5) point out minor thermic optima in the last glacial phase (see figure 7). This means good conditions for N_2-fixer-organisms activity, allowing shrubs and maybe trees to grow in the higher settlement (Liñares).



Isotopic Stage	Macropaleontological data	Coastal sedimentary data	Glacial data: Sedimentary	Glacial data: Geomorphological
1 13,000	*Homo sapiens sapiens* (210 ± 70 BP) **Arcoia** Ursus arctos (7,460 ± 95 BP) **Tarelo**	(1,795 ± 75 BP) ***Doniños*** *(1)* (3,970 ±50 & 4,350 ±90 BP) ***Seselle*** *(2)* (4,135 ± 80 BP) ***Puerta Real*** *(2)* (12,275 ± 95 BP) ***Ares*** *(2)*	***(8,990 ± 400 BP) Lucenza*** *(3)*	
2 28,000	*Cervus elaphus* (17,720 ± 185 BP) **Liñares** *Ursus spelaeus* (24.090 ± 440 BP) **Eirós** *Speleothem* (28,233 ± 5.027 BP) **Eirós**		***(13,400±400 BP) Laguna Grande*** *(3)* ***(23,300±400 BP) Villaseca*** *(3)* ***(25,000 BP) La Mata*** *(3)*	***Inner drumlin*** ***15,465 ± 6,991 BP (Q4) Queixa*** ***Outer drumlin*** ***21,646 ± 16,963 BP (Q3) Queixa***
3 60,000	*Ursus spelaeus* (35,230 ± 1.430 BP) **A Ceza** *Ursus spelaeus* (35,000 ± 1.440 BP) **Liñares** *Ursus spelaeus* (>38,000 BP) **Liñares** *Cervus elaphus* (>38,000 BP) **Liñares**	***Slope series:*** (37,505 ± 1.925 BP) ***Sorrizo*** (38,000 BP) ***Leira*** (>38,000 BP) ***Rañal***		***Frontal moraine complex:*** ***Chaguazoso (Queixa)*** ***Lagoa de Marinho (Gerês)*** ***Prada-II (Queixa)***
4 75,000	***Speleothem (97,051 ± 15,426 BP) Eirós***			
a 85,000				
b 92,000				
5 c				
d 115,000				
5 e 130,000	***Speleothem (117,252 ± 75,438 BP) Eirós***			***Frontal moraine complex*** ***126,184 ± 13,260 BP (Q2) Queixa*** ***Difluent valley*** ***130,732 ± 16,838 BP (AX1) Xurés***
6				***Moraine complex from:*** ***Fafião (Gerês)*** ***Fecha (Gerês, Xurés)*** ***Vilamés (Gerês, Xurés)*** ***Castiñeiras y Prada-I (Queixa)***

Figure 7.- Paleoclimatic reconstruction for Late Pleistocene in NW Iberian Peninsula. On the left, global thermic reference curve from Vostok ice core (modified from JOUZEL et al., 1987 In ANDERSEN & BORNS, 1994). Data from different subjects agree when defining changes in paleoenvironmental conditions (FERNÁNDEZ, 1999; VILA, 1999).[1] Lagoon, [2] Ria bottom series, [3] Glacial lake.

More or less 38-40,000 y BP, climate would be in a *warm/soft* short phase (inside a general cooling corresponding to the last glacial phase, see figure 7). At this moment, around Liñares cave could exist a kind of C3 vegetation which is now reflected, as basement of trophic chain, in *Ursus spelaeus* signals. As signal $\delta^{15}N$ is lower than Eirós one, we understand that atmospheric-nitrogen fixation should be very active due to better conditions for this process. Soil acidity and soil age, with influence in soil $\delta^{15}N$ are equivalent in both sites. Non-suckling $\delta^{15}N$ data agree with colder conditions: limitation in N2-fixers activity when parameters as temperature and available liquid water decrease.

Liñares site is demonstrated as *Ursus spelaeus* refuge around 35,000 yBP (GRANDAL & LÓPEZ, 1998). Its occupation does not correspond with Eirós one (approximately 25,000 yBP). The former felt more influence of glacial conditions (even when both were out of glacial-ice-limits) because of its altitude and location (GRANDAL et al., 1997). It is consistent to suppose that when weather conditions were so hard to prevent a normal activity of cave bears -we guess around 25,000 yBP- they would descend from sites as Liñares to easier stuffs as Eirós.

As figure shows 7, Liñares occupation (40,000 yBP aprox.) would be located inside Isotopic Stage 3. That age coincides with a wet climate, *warm* enough to allow the growing of significant taxa in mountains. On the other side, Eirós term would belong to Isotopic Stage 2, when all references show a hardening in conditions at the highest locations of the Courel Sierra. The *soft* phase in Isotopic Stage 3 agree with some polinic records at lower altitude (FERNÁNDEZ et al., 1991 and RAMIL, 1992 *In* PÉREZ & RAMIL, 1996).

CONCLUSIONS

Isotopic biochemistry data support the theory of herbivore diet for *Ursus spelaeus* due to low $\delta^{15}N$ and $\delta^{13}C$ values.

This isotopic study does not consider other factors than environmental ones due to sample selection (see *Sample and Techniques*). The studied sample corresponds to different sites, Liñares and Eirós, with a non contemporaneous animal occupation (GRANDAL & VIDAL, 1997; LÓPEZ & GRANDAL, 1998). We understand the significant differences in $\delta^{15}N$ as caused by environmental changes.

As N_2 fixation raises during a *warm* event included in a glacial period, it can be identified a a warm phase around 40,000 yBP (Isotopic Stage 3) included in the last glacial event for NW Iberian Peninsula. Then, caves as Liñares (1,115 m.a.s.l.) were occupied by *Ursus spelaeus*. Around 25,000 y BP (Isotopic Stage 2) the climate changed towards colder condi-

tions and individuals moved to lower sites as Eirós (780 m.a.s.l.).

Global references as the Vostok ice core prove that the alternation -cold and warm phases- pattern included in the last glacial event and inferred by the paleontological isotopic record of Eirós and Liñares is feasible.

Aknowledgements

This paper is a contribution to the Research Project XUGA10308A97. *Laboratorio Xeolóxico de Laxe* has provided with a high valuable sample in order to carry this work out. Juan Ouro was really patient with our computer doubts.

REFERENCES

ANDERSEN, B.G. & BORNS, H.W. Jr. (1994). *The Ice Age World. An introduction to Quaternary History and Research with Emphasis on North America and Northern Europe during the last 2,5 Million Years*. Scandinavian University Press, Oslo.

ANDERSON, T. (1992). *Black Bear. Seasons in the Wild*. Voyageur Press, Inc. Minnesota.

AMBROSE, S.H. (1991). Effects of Diet, Climate and Physiology on Nitrogen Isotope Abundances in Terrestrial Foodwebs. *Journal of Archaeological Science*, 18: 293-317.

AMBROSE, S.H. & DE NIRO, M.J. (1989). Climate and Habitat Reconstruction Using Stable Carbon and Nitrogen Isotope Ratios of Collagen in Prehistoric Herbivore Teeth from Kenya. *Quaternary Research*, 31: 407-422.

BOCHERENS, H.; FIZET, M.; MARIOTTI, A.; LANGE-BADRE, B.; VANDERMEERSCH, B.; BOREL J.P. & BELLON G. (1991a). Isotopic biogeochemistry ($\delta^{13}C$, $\delta^{15}N$) fo fossil vertebrate collagen: application to the study of a past food web including Neandertal man. *Journal of Human Evolution*, 20: 481-492.

BOCHERENS, H.; FIZET, M.; MARIOTTI, A.; BILLIOU, D.; BELLON, G.; BOREL, J.P. & SIMONE, S. (1991b). Biogéochimie isotopique ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$) et paléoécologie des ours pléistocènes de la grotte d'Aldène. *Bulletin du Museé Antropologuique et Prehistorique. Monaco*, 36: 29-49.

BOCHERENS, H.; FIZET, M. & MARIOTTI, A. (1994). Diet, physiology and ecology of fossil mammals as inferred from stable carbon and nitrogen insotope biogeochemistry: implications for Pleistocene bears. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 107: 213-225.

BOCHERENS, H.; FOGEL, M.L.; TUROSS, N. & ZEDER, M. (1995). Trophic structure and Climatic Information from isotopic signatures in Pleistocene cave fauna of Southern England. *Journal of Archaeological Science*, 22: 327-340.

BOCHERENS, H. (1997). Alimentation des ours et signatures isotopiques. *L'homme et l'ours/Man and bear Internation Meeting*, 4-6 Nov. GIRPPA. Grenoble.

BOCHERENS, H.; TRESSET, A.; WIEDEMANN, F.; GILIGNY, F.; LAFAGE, F.; LANCHON & MARIOTTI, A. (1997a). Diagenetic evolution of mammal bones in two French Neolithic sites. *Bulletin de la Sociète Géologuique de France*, 168 (5): 555-564.

BOCHERENS, H.; BILLIOU, D.; PATOU-MATHIS, M.; BONJEAN, D.; OTTE, M. & MARIOTTI, A. (1997b). Paleobiological implications of the isotopic signatures ($\delta^{13}C$, $\delta^{15}N$) of fossil mammal collagen in Scladina Cave (Sclayn, Belgium). *Quaternary Research*, 48: 370-380.

CORMIE, A.B. & SCHWARCZ, H.P. (1994). Stable isotopes of nitrogen and carbon of North American white-tailed deer and implications for paleodietary and other food web studies.

Plaeogeography, Palaeoclimatology, Palaeoecology, 107: 227-241.

DE NIRO, M.J. (1985). *Post mortem* preservation and alteration of *in vivo* bone collagen isotope ratios in relation to palaeodietary reconstruction. *Nature*, 317: 806-809.

FERNÁNDEZ MOSQUERA, D. (1998). Biogeoquímica isotópica ($\delta^{13}C$, $\delta^{15}N$) del *Ursus spelaeus* del yacimiento de Cova Eirós, Lugo. *Cad. Lab. Xeol. Laxe*, 23: 237-249.

FERNÁNDEZ MOSQUERA, D. (1999). *Aplicación de los nucleidos cosmogénicos a la datación de procesos geológicos*. Tesis de licenciatura. Universidade da Coruña, 114 pp. No published.

GRANDAL d'ANGLADE, A. (1993) *Estudio paleontológico de los restos de Ursus spelaeus ROSENMÜLLER-HEINROTH 1794 (Mammalia, Carnivora, Ursidae) de Cova Eirós (Triacastela, Lugo, NW de la Península Ibérica)*. Tesis Doctoral. Universidade da Coruña, 256 pp.

GRANDAL d'ANGLADE, A. & VIDAL ROMANÍ, J.R. (1997). A population study on the Cave Bear (*Ursus spelaeus* ROS.-HEIN.) from Cova Eirós (Triacastela, Galicia, Spain). *Geobios*, 30 (5): 723-731.

GRANDAL d'ANGLADE, A.; LÓPEZ-GONZÁLEZ, F. & VIDAL ROMANÍ, J.R. (1997). Condicionantes en la distribución de macromamíferos en Galicia (NW Península Ibérica) durante el Cuaternario superior. *Cad. Lab. Xeol. Laxe*, **22**: 43-66.

GRANDAL d'ANGLADE, A & LÓPEZ-GONZÁLEZ, F. (1998). A population study on the Cave Bears (*Ursus spelaeus* Rosenmüller-Heinroth) from Galician caves, NW of Iberian Peninsula. *Cad. Lab. Xeol. Laxe*, 23: 215-224.

IACUMIN, P.; BOCHERENS, H.; DELGADO HUERTAS, A.; MARIOTTI, A. & LONGINELLI, A. (1997). A stable isotope study of fossil mammal remains from the Paglicci cave, Southern Italy. N and C as palaeoenvironmental indicators. *Earth and Planetary Sciencie Letters*, 148: 349-357.

KOCH, P.L.; FOGEL, M.L. & TUROSS, N. (1994). Tracing the diets of fossil animals using stable isotopes. *In*: *Stable Istopes en Ecology and Environmental Science. Lajtha, K. & Michener, R.H. (Eds.) Methods in Ecology Serie*. Blackwell Scientific Publications

KURTÉN, B. (1976). *The Cave Bear Story. Life and Death of a Vanished Animal*. Columbia University Press. New York.

LAJTHA, K. & MICHENER, R.H. (1994). Introduction. *In*: *Stable Isotopes in Ecology and Environmental Science*. Lajtha & Michener Eds. Methods in Ecology Serie. Blackwell Scientific Publications.

LAJTHA, K. & MARSHALL, J.D. (1994) Sources of variation in the stable isotopic composition of plants. *In*: *Stable Istopes in Ecology and Environmental Science*. Lajtha, K. & Michener, R.H. (Eds.) Methods in Ecology Serie. Blackwell Scientific Publications.

LÓPEZ GONZÁLEZ, F. (1996). *Estudio geomorfológico y paleontológico del endocarst de Liñares S (Lugo)*. Tesis de Licenciatura. Universidade da Coruña, 90 pp. No published.

LÓPEZ GONZÁLEZ, F.; GRANDAL d'ANGLADE, A. & VIDAL ROMANÍ, J.R. (1997). Análisis tafonómico de la

muestra ósea de Liñares sur (Lugo, Galicia). *Cadernos do Laboratorio Xeolóxico de Laxe*, 22: 67-80.

LÓPEZ GONZÁLEZ, F. & GRANDAL d'ANGLADE, A. (1998). Datos sobre *Cervus elaphus* (Cervidae, Artiodactyla, Mammalia) en cavidades cársticas de Galicia (NW España). *Cadernos do Laboratorio Xeolóxico de Laxe*, 23: 201-213.

MILTON, J.S. (1994). *Estadística para Biología y Ciencias de la Salud. 2ª edición castellana*. Ed. McGraw-Hill-Interamericana de España. Madrid.

NELSON, R.A.; JONES, J.D.; WAHNER, H.W.; MCGILL, D.B. & CODE, C.F. (1975). Nitrogen metabolism in bears: urea metabolism in summer starvation and in winter sleep and role of urinary bladder in water and nitrogen conservation. *Mayo Clinic Pro.*, 50: 141-146.

PÉREZ ALBERTI, A. & RAMIL REGO, P. (1996). La evolución bioclimática y sus consecuencias: el ejemplo de los paleopaisajes del Cuaternario en Galicia. *Gallaecia* 14/15: 31-66.

STOTT, A.W. & EVERSHED, R.P. (1996). $\delta^{13}C$ Analysis of Cholesterol Preserved in Archaeological Bones and Teeth. *Analitical Chemistry*, 68: 4402-4408.

TURBAN-JUST, S. & SCHRAMM, S. Stable carbon and nitrogen isotope ratios of individual aminoacids give new insights into bone collagen degradation. *Bulletin de la Sociètè Géologuique de France*, 169 (1): 109-114.

TUROSS, N. & STATHOPLOS, L. (1993). Ancient proteins in fossil bones. *Methods in Enzymology*, 224: 121-129.

VIDAL ROMANÍ, J.R. (1996). Geomorfología de Galicia. *In*: Historia de Galicia. Tomo de Xeografía. *Hércules de Ediciones*. A Coruña, pp: 7-67.

VILA TABOADA, M. (1998). Nota sobre el estudio de ADN antiguo en restos óseos de macromamíferos cuaternarios de Galicia. *Cadernos do Laboratorio Xeolóxico de Laxe*, 23: 263-270.

VILA TABOADA, M. (1999). *Paleontología molecular: Nuevas herramientas para el estudio de restos óseos de macromamíferos cuaternarios*. Tesis de licenciatura. Universidade da Coruña, 236 pp. No published.

WANG, Y. & CERLING, T.E. (1994). A model of fossil tooth and bone diagenesis: implications for paleodiet reconstruction from stable isotopes. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 107: 281-289.